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Results of the Interlaboratory Method Validation Study for Determination of *Cryptosporidium* and *Giardia* Using U.S. EPA Method 1623

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Disclaimer

The content of this report version is identical to the April 1999 version of *Results of the Interlaboratory Method Validation Study for Determination of* Cryptosporidium *and* Giardia *Using U.S. EPA Method 1623.*

This summary report has been reviewed by the Analytical Methods Staff in the Engineering and Analysis Division within the EPA Office of Water. Mention of company names, trade names, or commercial products in this report does not constitute endorsement or recommendation for use.

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You may obtain copies of *Results of the Interlaboratory Method Validation Study for Determination of* Cryptosporidium *and* Giardia *Using U.S. EPA Method 1623* through the U.S. EPA National Service Center for Environmental Publications (NSCEP), 11029 Kenwood Road, Cincinnati, OH 45242; telephone: 513-489-8190; internet: http:/www.epa.gov/ncepi.

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Executive Summary

This report presents the results of the U.S. Environmental Protection Agency's (EPA's) interlaboratory validation study (the "Study") of Method 1623: *Cryptosporidium* and *Giardia* by Filtration/IMS/FA (the "Method"). The purpose of this Study was to determine the precision and recovery for *Cryptosporidium* and *Giardia* in reagent water and raw surface water matrices in multiple laboratories using the Method.

One referee laboratory and 11 participating laboratories were involved in the Study. The Study was conducted in February 1999. The referee laboratory used a flow cytometer to sort a known number of unstained *Cryptosporidium* oocysts and unstained *Giardia* cysts into spiking suspension containers. Six single-blind spiking suspensions and one double-blind reagent water blank were distributed to each participating laboratory. Each spiking suspension contained approximately 129 oocysts and 129 cysts.

Each laboratory analyzed four spiked reagent water samples, one reagent water blank, two spiked raw surface water samples, and one unspiked raw surface water sample according to the December 1998 version of the Method, as amended by technical clarifications. These clarifications have been incorporated in the final version of the Method.

Sample results submitted by the laboratories were validated using a standardized data review process to verify that results were generated in accordance with Method and Study specifications. A summary of the overall precision and recovery for Method 1623 is provided in **Table 1**. This summary includes all valid results, before outlier analyses, and account for background protozoa levels in the raw surface water samples, as environmental *Cryptosporidium* oocysts and *Giardia* cysts were detected by the Method in several unspiked samples.

Table 1. Summary Results for Method 1623

Matrix	Organism	Mean Recovery	Mean RSD or RPD
Reagent water	*Cryptosporidium*	40%	24%
	Giardia	38%	29%
Source water	*Cryptosporidium*	38%	36%
	Giardia	42%	32%

There was no statistically significant difference between the *Cryptosporidium* results and the *Giardia* results using Method 1623. In addition, the *Cryptosporidium* results using Method 1623 in this Study were not statistically different from the *Cryptosporidium* results using Method 1622 during a similar study conducted in August 1998.

Laboratories and individual results disparate from the average results produced by all laboratories were eliminated from consideration through outlier analysis according to published American Society for Testing and Materials (ASTM) procedures. The final QC acceptance criteria for simultaneous *Cryptosporidium* and *Giardia* analyses developed for the Method are listed in **Table 2**.

Table 2. Final Method 1623 Quality Control Acceptance Criteria

Performance test	Acceptance criteria	
	Cryptosporidium	***Giardia***
Initial precision and recovery (IPR)		
Mean recovery	21% - 100%	17% - 100%
Precision (as maximum relative standard deviation)	40%	41%
Ongoing precision and recovery (OPR)	19% - 100%	16% - 100%
Matrix spike/matrix spike duplicate (MS/MSD)		
Mean recovery	13% - 111%	15% - 118%
Precision (as maximum relative percent difference)	61%	30%

Based on the results of this Study, U.S. EPA Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA (EPA-821-R-99-006) is considered valid for use in determining the concentration of *Cryptosporidium* oocysts and *Giardia* cysts in water.

SECTION 1 BACKGROUND

To support future regulation of protozoa in drinking water, the Safe Drinking Water Act Amendments of 1996 require the U.S. Environmental Protection Agency (EPA) to evaluate the risk to public health posed by drinking water contaminants, including waterborne parasites, such as *Cryptosporidium* and *Giardia.* To implement these requirements, EPA must accurately assess *Cryptosporidium* and *Giardia* occurrence in raw surface waters used as source waters for drinking water treatment plants, determine drinking water treatment and disinfection process needs, and set meaningful protozoa standards for drinking water. Method 1623 was developed to support EPA's assessment of *Cryptosporidium* and *Giardia* occurrence in raw surface waters.

1.1 History of Method Development

EPA initiated an effort in 1996 to identify new and innovative technologies for protozoan monitoring and analysis. After evaluating potential alternatives to the then-current method through literature searches, discussions with research and commercial laboratories, and meetings with experts in the field, the Engineering and Analysis Division within the Office of Science and Technology within EPA's Office of Water developed draft Method 1622 for *Cryptosporidium* detection in December 1996. The draft method was revised in January, May, and December 1997, based on comments from multiple peer reviews and two single-laboratory validation studies.

Because development of an acceptable immunomagnetic separation system for *Giardia* lagged behind development of an acceptable system for *Cryptosporidium*, and because a reliable method for *Cryptosporidium* detection was urgently needed, EPA chose not to delay an interlaboratory validation of the *Cryptosporidium*-only method by waiting for the *Giardia*-detection capability. Method 1622 for *Cryptosporidium* was validated through an interlaboratory study in August 1998, and was revised as a final, valid method for detecting *Cryptosporidium* in water in January 1999.

Final tests on the performance of an acceptable, combined *Cryptosporidium/Giardia* IMS system were conducted in October 1998 at multiple laboratories on reagent water and raw surface water. Based on the results of these tests, EPA decided to move forward with interlaboratory validation of the full method for combined detection of both organisms. To avoid confusion with Method 1622, which already had been validated and was in use both domestically and internationally as a stand-alone *Cryptosporidium*-detection method, EPA designated the new combined procedure Method 1623.

EPA conducted the interlaboratory validation study of Method 1623 in February 1999 to characterize the precision and recovery of the Method in reagent water and raw surface water at 11 laboratories. This report describes the design, results, and conclusions of this Study.

1.2 Summary of Method

A 10-L water sample is filtered in the laboratory and the oocysts, cysts, and extraneous materials are retained on the filter. Materials on the filter are removed by elution with an aqueous buffered salt and detergent solution. The eluate is centrifuged to concentrate the eluted particles into a pellet, and the supernatant fluid is aspirated. The oocysts and cysts are magnetized by attachment of magnetic beads conjugated to either anti-*Cryptosporidium* or anti-*Giardia* antibodies. The magnetized oocysts and cysts are separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts and cysts, and the organisms are applied to a well slide. The oocysts and cysts are stained on the slide with fluorescently labeled monoclonal

antibodies and vital dye. The stained oocysts are examined and enumerated using fluorescence and differential interference contrast (D.I.C.) microscopy.

SECTION 2 STUDY DESIGN AND OBJECTIVES

The following objectives were established for the interlaboratory (round-robin) validation study of the precision and recovery of Method 1623:

- Meet the method validation requirements established for use in EPA's *Guide to Method Flexibility and Approval of EPA Water Methods*[1] (performance-based measurement system (PBMS) Tier 2 requirements)
- Determine the performance capabilities of Method 1623
- Establish QC acceptance criteria for performance tests in the Method
- Ensure that all samples and data produced under the Study were generated according to the analytical and QA/QC procedures in the current revision of the Method

To accomplish these objectives, the Study was designed in three steps. Step 1 involved identifying the laboratories required for the Study. Step 2 involved preparing spiking suspensions with known levels of *Cryptosporidium* oocysts and *Giardia* cysts, and distributing these spiking suspensions to laboratories participating in the study. Step 3 involved analysis of reagent and raw surface water samples by the participant laboratories and submission of the data from these analyses. The reagent and raw surface water samples analyzed in this step were spiked with the spiking suspension prepared in Step 2 to create samples with oocyst and cyst concentrations that were known to EPA, but not known to the participant laboratories.

2.1 Identification of Laboratories

Two types of laboratories were required for the Study: a referee laboratory to prepare and distribute enumerated spiking suspensions, and participant laboratories to analyze the samples and provide EPA with data on Method performance. Sections 2.2.1 and 2.2.2 provide details on how both types of laboratories were identified for the Study.

2.1.1 Referee Laboratory

EPA's primary criterion for selecting the referee laboratory was a demonstrated ability to accurately and precisely prepare spiking suspensions containing fewer than 500 organisms using a flow cytometer. This criterion was based on data indicating that flow cytometry was a more precise means of enumerating protozoa for spiking suspensions than other techniques, such as hemacytometer chamber counting and well slide enumeration. This criterion also was based on the results of the Method 1622 interlaboratory validation study, in which the referee laboratory successfully generated very precise spike doses using a flow cytometer. The Wisconsin State Laboratory of Hygiene, which had demonstrated expertise in using flow cytometry on environmental samples and enumerating protozoa using flow cytometry, was used as the referee laboratory for this Study.

2.1.2 Participant Laboratories

The criteria for selecting the participant laboratories was a demonstrated ability to perform epifluorescence microscopy–based protozoa detection methods and to perform the techniques required in Method 1623. These criteria were established to ensure that the laboratories participating in the study were qualified to perform protozoan analyses on environmental samples, and were sufficiently familiar

[1]EPA Guide to Method Flexibility and Approval of EPA Water Methods, EPA 821-D-96-004, December 1996.

with Method 1623 to ensure that a learning experience was not included in the statistics of the collaborative study, as per American Society for Testing and Materials guidance[2]. With one exception, the participant laboratories in this Study were the same as those participating in the Method 1622 interlaboratory validation study, because EPA had issued contracts to these laboratories for all aspects of the method validation process. (Although the Wisconsin State Laboratory of Hygiene participated in the Method 1622 interlaboratory validation study, this laboratory was prohibited from participating in this Study because of their role as referee laboratory.) Demonstration requirements were met by all participant laboratories through their participation in the Method 1622 validation study.

2.2 Preparation of Spiking Suspensions

The referee laboratory was required to prepare spiking suspensions using flow cytometry and to conduct tests to confirm the number of oocysts and cysts added to each sample. These tests allowed determination of the following:

- Spiking suspension transfer efficiencies
- Mean spike level, based on ongoing calibration samples
- Trip effects on the spiking suspensions

In addition to spiking suspensions containing oocysts and cysts, the Study design also called for the preparation of reagent water blank samples containing no oocysts or cysts in an oocyst- and cyst-free environment at the referee laboratory. These reagent water blank samples were to be labeled in a manner identical to the spiking suspensions to enable them to be used as double-blind blanks.

2.3 Analysis of Water Samples

The following objectives were established for analysis of water samples:

- Generate precision and recovery data on reagent water and raw surface water
- Generate data that meet EPA Tier 2 PBMS method validation requirements
- Generate data to assess intralaboratory variability
- Generate data to assess interlaboratory variability

To meet these objectives, the Study was designed to include at least three laboratories, each of which would analyze reagent water and raw surface water. Reagent water was analyzed to provide a means for assessing the performance of Method 1623 on a matrix that could be duplicated in each laboratory in the study and duplicated in the future. Raw surface water samples were analyzed to provide a means for assessing the performance of Method 1623 on waters comparable to the raw surface waters that would be analyzed during the Supplemental Surveys (a 12-month-long national survey on protozoa occurrence in drinking water utility source waters for which Method 1623 was developed). Details on how reagent water and raw surface water samples were to be used in the Study are provided below in Sections 2.3.1 and 2.3.2.

2.3.1 Reagent Water Sample Analysis

Each participant laboratory was required to analyze five, 10-L reagent water samples. Each laboratory was instructed to spike each of the five reagent water samples with spiking suspensions provided by the referee laboratory. Four reagent water samples were spiked with single-blind spiking suspensions

[2]Standard Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D-19 on Water (ASTM D2777-96), September 1996.

containing oocysts and cysts; the fifth reagent water sample was spiked with reagent water that was sent in a tube that was indistinguishable from the suspension tubes containing protozoa.

Analysis of these five reagent water samples by at least three participant laboratories was designed to provide EPA with the following:

- An indication of method performance in the absence of interfering materials
- Identification of laboratory contamination through analysis of the double-blind reagent water blank sample
- An indication of intralaboratory performance through analysis of four replicate samples
- An indication of interlaboratory performance through analysis of identical samples by multiple laboratories
- Sample results required for PBMS Tier 2 method validation
- Sufficient data to develop initial precision and recovery (IPR) and ongoing precision and recovery (OPR) quality control (QC) acceptance criteria for each organism

2.3.2 Raw Surface Water Sample Analysis

In addition to analyzing the reagent water samples described above, each participant laboratory was required to analyze three, 10-L raw surface water samples. Each laboratory was required to collect all three raw surface water samples from the same source, and this source was to be a surface water body serving as the source water for a drinking water utility. Each participant laboratory was instructed to spike two of the three raw surface water samples with spiking suspensions received from the referee laboratory. The third raw surface water sample was to be analyzed unspiked. Each laboratory also was required to supply the turbidity of the samples with their results.

Analysis of these three raw surface water samples by at least three participant laboratories was designed to provide EPA with the following:

- An indication of method performance using a wide range of raw surface waters (a different raw surface water for each laboratory)
- Identification of detectable oocysts and cysts present in the surface water collected for the Study (unspiked sample)
- The sample results required for PBMS Tier 2 method validation
- Sufficient data to develop matrix spike/matrix spike duplicate (MS/MSD) QC acceptance criteria for Method 1623

Due to potential matrix effects, the raw surface water sample results would not be used to assess laboratory performance.

2.3.3 Data Reporting

Each laboratory was required to submit data on standardized bench sheets and report forms designed for use with Method 1623.

SECTION 3 STUDY IMPLEMENTATION

3.1 Study Management

The Study was designed and managed by the Analytical Methods Staff (AMS) within the EPA Office of Water's Engineering and Analysis Division. Day-to-day coordination of activities under the Study was performed by the Sample Control Center (SCC) operated by DynCorp I&ET. Referee laboratory activities were performed by the Wisconsin State Laboratory of Hygiene, operating under the direction of SCC.

3.2 Laboratory Participants

The 11 participating laboratories and the referee laboratory involved in the Study are listed in **Table 3**.

Table 3. Laboratories Participating in the Method 1623 Interlaboratory Validation Study[1]

Analytical Services, Inc. P.O. Box 515 50 Allen Brook Lane Williston, VT 05495	**Milwaukee Health Department** 841 N. Broadway, Rm. 308 Milwaukee, WI 53202
CH Diagnostic 214 SE 19th Street Loveland, CO 80537	**New York City DEP** Sutton Park, 465 Columbus Ave Valhalla, NY 10595
City of Pittsburgh 6433 Forward Avenue Pittsburgh, PA 15217	**NSF International** 3475 Plymouth Road Ann Arbor, MI 48105
Clancy Environmental Consultants P.O. Box 314 St. Albans, VT 05478	**Upper Mohawk Valley Regional Water Board** One Kennedy Plaza, 3rd Floor Utica, NY 13502
Grants Pass Water Laboratory, Inc. 558 NE F Street, Suite 1 Grants Pass, OR 97526	**U.S. EPA Region 10 Manchester Environmental Laboratory** 7411 Beach Drive Port Orchard, WA 98366
Metropolitan Water District of Southern California 700 Moreno Avenue La Verne, CA 91750-3399	
Referee laboratory:	**Wisconsin State Laboratory of Hygiene** 2601 Agriculture Drive Madison, WI 53718

[1]No endorsement of these laboratories is implied, nor should any be inferred. Participant laboratories have been randomly assigned numbers from 1 to 11 for purposes of presenting data in this report.

3.3 Tier Level

This Study meets or exceeds the EPA performance-based measurement system (PBMS) Tier 2 method validation requirements for development of quality control (QC) acceptance criteria for initial precision and recovery (IPR), ongoing precision and recovery (OPR), and matrix spike/matrix spike duplicate (MS/MSD) tests, as set forth in Table 4-2 of EPA's *Guide to Method Flexibility and Approval of EPA*

Water Methods[3]. A comparison of the PBMS Tier 2 requirements and the design of this Study is presented in **Table 4.**

Table 4. Comparison of PBMS Tier 2 Validation Requirements and this Validation Study

PBMS Tier 2 Requirements	Components of this Study
3 participant laboratories	11 participant laboratories
1 matrix type plus reagent water	1 matrix type (raw surface water) plus reagent water
3 public water systems	11 public water systems
12 IPR samples (4 replicate sample analyses in 3 laboratories)	44 IPR samples (4 replicate analyses in 11 laboratories)
6 MS/MSD samples (1 MS and 1 MSD sample in 3 laboratories)	22 MS/MSD samples (1 MS and 1 MSD sample in 11 laboratories)

3.4 Schedule

The Study schedule is provided in **Table 5.**

Table 5. Sequence of Events for the Interlaboratory Validation Study of Method 1623

Date	Event
January 11, 1999	*Cryptosporidium* oocysts collected from bovine host
February 5, 1999	Participant laboratories notified of round-robin schedule
February 6, 1999	*Giardia* cysts collected from gerbil host
February 15, 1999	Referee laboratory prepares and ships spiking suspensions
February 15 - 26, 1999	Participant laboratories collect source water samples, receive spiking suspensions, and conduct analyses
March 16, 1999	Last set of sample results received by SCC

3.5 Sample Matrices

Two sample matrix types were used in the Study: reagent water and raw surface water. Each participant laboratory provided its own reagent water and raw surface water. Raw surface water samples were collected as grab samples from a surface water body serving as the source for a drinking water utility. All raw surface waters used in the Study were collected in February 1999.

3.6 Sample Preparation and Distribution

The Wisconsin State Laboratory of Hygiene performed the following sample preparation and distribution tasks:

- Initial calibration of the flow cytometer to ensure accurate and precise sorting of oocysts and cysts
- Preparation of spiking suspensions for distribution to Study participants and ongoing calibration of flow cytometer
- Distribution of spiking suspensions to Study participants

[3]EPA Guide to Method Flexibility and Approval of EPA Water Methods, EPA 821-D-96-004, December 1996.

- Analysis of trip control samples

These tasks, as well as details on the source of the oocysts and cysts used in the study, are described in Sections 3.6.1 through 3.6.5, below.

3.6.1 Source of *Cryptosporidium* Oocysts and *Giardia* Cysts Used in Study

Cryptosporidium parvum oocysts from the Harley Moon strain were used in the Study. The oocyst stock suspension was obtained from Marilyn Marshall of the University of Arizona's Department of Veterinary Science. The suspension consisted of approximately 1 x 10^7 *Cryptosporidium parvum* oocysts in a 0.01% Tween-20 solution containing 100 U of penicillin, 100 μg of streptomycin, and 100 μg of gentamicin sulfate per mL. The oocysts were shed and collected from a bovine host on January 11, 1999, and purified using discontinuous sucrose and cesium chloride centrifugation gradients.

Giardia intestinalis cysts from the CH3 strain were used in the Study. The cyst stock suspension was obtained from PRL DynaGenics. The suspension consisted of approximately 5 x 10^6 *Giardia intestinalis* cysts in a 0.01% Tween-20 solution containing 100 U of penicillin, 100 μg of streptomycin, and 100 μg of gentamicin sulfate per mL. The cysts were shed and collected from a gerbil host on February 6, 1999, and purified using a $ZnSO_4$ underlay procedure.

The referee laboratory used the oocysts and cysts from the stock suspensions described above to prepare blind, enumerated spiking suspensions for distribution to the Study participants and to prepare spiking suspensions for use in calibration tests and trip control tests. All spiking suspensions were prepared using unstained oocysts and cysts that were sorted through an EPICS Elite flow cytometer into 50-mL polypropylene, screw-cap tubes containing approximately 40 mL of reagent water plus 0.01% Tween-20. The volume added by the flow cytometer is approximately 25 μL.

The flow cytometer in the Study sorts organisms from a stock suspension until the instrument detects that the target value of organisms (130 for all suspensions in the Study) have been sorted into the collection tube (the spiking suspension tube) When the target value is reached, the instrument's sorting mechanism is automatically disabled, and the flow of organisms is directed to waste, rather than the collection tube. The flow is manually stopped before the spiking suspension tube is removed from the collection area.

3.6.2 Initial Calibration of Flow Cytometer

To calibrate the flow cytometer, oocysts and cysts were stained in suspension with CelLabs fluoroisothiocyanate-labeled anti-*Cryptosporidium* and anti-*Giardia* antibodies. Suspension staining entailed adding 10 μL of CelLabs anti-*Cryptosporidium* antibodies to 10 μL oocyst stock suspension, and adding 50 μL of CelLabs anti-*Giardia* antibodies to 100 μL of cyst stock suspension. The suspensions were vortexed and incubated at room temperature for 30 minutes. The stained suspensions were sorted through the flow cytometer onto well slides. The stained oocysts and cysts were identical to the unstained organisms and came from the same stock suspensions. Prestaining allowed direct reading of the well slides without subjecting them to multiple rinses that could cause loss of the organisms.

The referee laboratory set the flow cytometer to sort the stained organisms based only on forward scatter and side scatter (i.e., the stained organisms were not sorted based on fluorescence). The flow cytometer settings for the initial calibration samples were identical to the settings used to sort the unstained organisms for distribution to Study participants.

Flow cytometer calibration consisted of sorting protozoa onto 20 consecutive well slides, 10 of which received oocysts and 10 of which received cysts. The flow cytometer was set to deliver 130 organisms.

After each sort, the referee laboratory added mounting medium and a cover slip, and enumerated each slide using epifluorescent microscopy.

The calibration samples were prepared on February 15 and counted on the same day. Results of calibration sample counts are provided in **Table 6.**

Table 6. Results of Initial Calibration

Sample no.	Oocysts counted	Cysts counted
1	130	133
2	131	128
3	126	127
4	127	130
5	129	134
6	129	133
7	132	131
8	127	129
9	127	130
10	127	131
Mean (± SD)	128.5 (± 2.0)	130.6 (± 2.3)

3.6.3 Preparation of Spiking Suspensions and Ongoing Calibration of Flow Cytometer

On February 17, 1999, the referee laboratory prepared seven spiking suspensions for distribution to each Study participant. Six suspensions were to contain 130 each oocysts and cysts, and one was to contain reagent water only.

To ensure that an accurate number of oocysts and cysts were sorted into the spiking suspension tubes, the referee laboratory prepared ongoing calibration samples throughout the spiking suspension preparation process. One calibration sample was prepared before and after every 10 spiking suspensions. The ongoing calibration samples were sorted directly onto well slides using stained organisms, and enumerated microscopically, as described in Section 3.6.2.

The referee laboratory generated a total of nine ongoing calibration samples. The order in which the ongoing calibration samples were run, and the counts for each calibration sample, are presented in **Table 7.** Based on the average of the ongoing calibration sample results, approximately 129 oocysts and cysts were sorted into each spiking suspension tube distributed to the participant laboratories.

Table 7. Ongoing Calibration Sample Results

Sample no.	Oocysts counted	Cysts counted
1	130	127
2	129	132
3	128	130
4	131	127
5	127	131
6	130	129
7	129	132
8	132	129
9	127	128
Mean (± SD)	129.2 (± 1.7)	129.4 (± 1.9)

Neither the target count of 130 oocysts and cysts nor the calculated mean spike level of approximately 129 oocysts and cysts were conveyed to the participant laboratories. The laboratories were aware that the suspensions contained a known number of oocysts and cysts. As a result, the spiking suspensions served as single-blind samples.

3.6.4 Distribution of Spiking Suspensions to Study Participants

In advance of sample distribution, SCC worked with each participant laboratory, the Centers for Disease Control (CDC) and the U.S. Department of Agriculture to secure permits for receipt of etiologic agents. All permits were approved and in place before sample preparation began.

After all spiking suspensions had been generated using the flow cytometer, and the reagent water blanks had been prepared, the referee laboratory labeled each tube with a number and a letter. Each laboratory was assigned a unique number, and was sent seven tubes labeled with that number plus a letter, from A to G. Samples labeled A - C and E - G contained oocysts and cysts, while samples labeled "D" contained no organisms. This information was not conveyed to the participant laboratories.

The referee laboratory packaged the tubes in compliance with CDC etiologic agent shipping regulations promulgated at 42 *CFR* part 72.3. These regulations require a packaging system that entailed: (1) shipping the spiking suspensions in a securely closed, watertight tube (the primary container), (2) placing the tubes in durable, watertight secondary container (a plastic, screwtop can), and (3) placing the secondary container and two ice packs in a specially labeled ice chest. The ice chest's outer box was pre-labeled to meet CDC specifications. The laboratory also completed the required Federal Express shipping forms, including a dangerous goods declaration, for shipment of etiologic agents.

Each box contained seven suspensions and was shipped via Federal Express on February 17, 1999, as a priority overnight delivery. However, dangerous goods specialists at Federal Express held up the shipment on February 17. The referee laboratory repacked the boxes with fresh ice packs and reshipped them on February 18. All samples were maintained at 0°C to 8°C during this period, and trip control results indicate no adverse effects on the suspensions. All but one participant laboratory received samples on February 19; the remaining laboratory received samples on February 20.

3.6.5 Trip Control Preparation and Analysis

While preparing the spiking suspensions for distribution to the participant laboratories, the referee laboratory prepared six additional suspensions at the same concentration for use as trip controls. Three of the suspensions contained unstained organisms, identical to the suspensions sent to the participant laboratories, and three contained stained suspensions, identical to the suspensions used for calibration tests of the flow cytometer. The referee laboratory shipped all six trip control suspensions to SCC via Federal Express priority overnight service at the same time the spiking suspensions were shipped to the participants laboratories. No trip control suspensions were stored at the referee laboratory.

The trip control suspensions shipped to SCC on February 18, 1999, were received on February 19. SCC personnel opened the box upon receipt, verified that the samples were cool and intact, and refrigerated the samples until February 22. On February 22, SCC added fresh freezer packs to the box, resealed the container, and shipped the samples to the referee laboratory via Federal Express priority overnight service.

The referee laboratory received the trip control samples from SCC on February 23, 1999, and stored the samples at 4°C until tests were conducted to determine whether sample shipment affected the transfer efficiency of oocysts and cysts from the sample tubes. On February 24, at the end of the seven-day period within which the laboratories were required to complete the concentration step for all samples, the referee laboratory poured the samples through a membrane filter, stained the three unstained samples with Meridian Merifluor fluoroisothiocyanate-labeled anti-*Cryptosporidium* and anti-*Giardia* antibodies, and enumerated all six trip controls using epifluorescent microscopy.

The trip control suspensions were transferred from the tubes according to the following procedure:

- 500 μL of of diluted Antifoam A (400 μL of Antifoam A in 100 mL of reagent water) was added to the spiking suspension tube.
- The tube was vortexed for 2 minutes.
- The suspension was poured from the tube through a 1-μm-pore-size, 25-mm-diameter polycarbonate membrane filter (Poretics Products, Livermore, CA, cat. no. 11057) supported by a polyester drain disc (Poretics Products cat. no. 09-753E).
- 20 mL of reagent water was added to the empty tube, which was then capped and vortexed for 10 seconds to rinse, then poured through the filter. This rinse was repeated using another 20 mL of reagent water.

The same procedure was used by the participant laboratories to transfer their spiking suspensions to 10-L sample carboys. Results of the transfer efficiency tests are provided in **Tables 8 and 9**.

Table 8. Results of Transfer Efficiency Trip Control Tests for *Cryptosporidium*

Sample no.	Spike level[1]	Disposition	Oocysts detected	Transfer efficiency
1	129.2 oocysts	Unstained	122	94.4%
2	129.2 oocysts	Unstained	129	99.8%
3	129.2 oocysts	Unstained	113[2]	87.4%
			Mean (± SD)	93.9% (± 6.2%)
4	129.2 oocysts	Stained	197	152.5%
5	129.2 oocysts	Stained	119	92.1%
6	129.2 oocysts	Stained	117	90.5%
			Mean (± SD)	Invalid[3]

[1]Spike level was determined from the results of the calibration samples prepared on February 17, 1999 (Table 6)
[2]Low recoveries may be due to incomplete vortexing of the vial at a volume of 40 mL
[3]Mean not calculated due to the invalid results for Sample 4

Table 9. Results of Transfer Efficiency Trip Control Tests for *Giardia*

Sample no.	Spike level[1]	Disposition	Oocysts detected	Transfer efficiency
1	129.2 oocysts	Unstained	131	101.2%
2	129.2 oocysts	Unstained	126	97.3%
3	129.2 oocysts	Unstained	117[2]	90.4%
			Mean (± SD)	96.3% (± 5.5%)
4	129.2 oocysts	Stained	124	95.8%
5	129.2 oocysts	Stained	125	96.6%
6	129.2 oocysts	Stained	123	95.0%
			Mean (± SD)	95.8% (± 0.8%)

[1]Spike level was determined from the results of the calibration samples prepared on February 17, 1999 (Table 6)
[2]Low recoveries may be due to incomplete vortexing of the vial at a volume of 40 mL

Although most organisms examined in the trip control tests exhibited fluorescence intensities of 3+ to 4+ (on a random 1+ to 4+ scale), some oocysts and cysts were 2+. This reduced fluorescence in some organisms could indicate trip stress.

More importantly, as noted in Table 8, the 197 oocysts counted in one of the prestained trip control samples, Sample 4, greatly exceeded the expected range of 127 to 131 oocysts. The reason for the high number of oocysts in this sample was not determined. Similarly high numbers of oocysts were detected in four additional samples at four different participant laboratories. No such problems were encountered with the *Giardia* spiking suspensions. Details on the samples analyzed by the participant laboratories, in which unexpectedly high numbers of oocysts were detected, are provided in Sections 5 and 6.

3.7 Sample Analyses by Participant Laboratories

Participant laboratories added spiking suspensions to reagent and raw surface water samples according to the Study design described in Section 2.3. The water samples were spiked and analyzed according to the December 1997 draft of Method 1622, as amended by the technical clarifications listed in Appendix A.

SECTION 4 DATA REPORTING AND VALIDATION

Participant laboratories submitted the following data to SCC for review and validation:

- Completed sample traffic reports
- Completed bench sheet for each sample
- Completed *Cryptosporidium* report form for each sample
- Completed *Giardia* report form for each sample
- Documentation of any additional information that would assist in evaluating the data

All 11 laboratories completed the Study and submitted data packages. Results were submitted for all 96 samples analyzed at the participant laboratories, yielding a 100% completion rate for the Study.

SCC used manual and automated data review procedures to check each data package for completeness and to review each sample result against requirements of the Study and Method 1623. Items reviewed for each sample included the following:

- Confirmation that original forms were submitted
- Confirmation that all holding times were met
- Confirmation that positive and negative staining controls were performed and acceptable
- Confirmation that all calculations were correct

Based on the SCC data review and subsequent discussions with the laboratories, the following data were considered invalid and unacceptable for inclusion in subsequent data analysis:

- The spiking suspensions received by Laboratory 3 arrived on Friday, February 19, and were left out, unrefrigerated, until Monday, February 22, as a result of an administrative error. The ice packs in the box were completely thawed and the suspensions had warmed to only slightly below room temperature. Both *Cryptosporidium* and *Giardia* exhibited poor morphological structure upon microscopic analysis. Because of concerns over the potential adverse impacts that the extended increase in temperature had on the organisms in the suspensions, all results from this laboratory were eliminated from subsequent data analysis.

- Laboratory 5 aspirated their centrifuged sample concentrates to a level very close to the surface of the pellet, resulting in potential organism losses that would not have occurred had a larger volume of eluent been left above the pellet. Perhaps more importantly, this laboratory experienced antibody staining problems with the lot of the Meridian Merifluor antibody kit used at their laboratory, resulting in underestimates of the number of organisms recovered from the sample and applied to the slide. The number of organisms counted on each slide as a result of D.I.C. examination greatly exceeded the number of organisms counted based on epifluorescence. Subsequent tests conducted by the laboratory confirmed that the antibody kit used by the laboratory did not stain a large percentage of organisms on a slide. All results from this laboratory were eliminated from subsequent data analysis.

- Laboratory 6 spilled the sample concentrate for Sample E during IMS, resulting in potential organism losses. The *Cryptosporidium* and *Giardia* data for that sample were eliminated from subsequent data analysis.

- Laboratory 9 did not add *Giardia*-IMS beads to any of their sample concentrates. All of the results from this laboratory were eliminated from subsequent data analysis because the sample results did not reflect simultaneous separation of *Cryptosporidium* and *Giardia*.

- The *Cryptosporidium* counts from one reagent water sample each in Laboratory 2 and Laboratory 10 and one raw surface water sample each in Laboratory 4 and Laboratory 8 exceeded the estimated number of organisms spiked by 31 to 239 organisms. This phenomenon also occurred in one of the trip control samples. The *Giardia* counts in these samples were not disparate from the counts in all other samples in the Study. The cause of the excessive number of oocysts in these five samples could not be determined by the referee laboratory, the participant laboratories, or SCC, but EPA does not believe that they were the result of contamination or other sample processing or examination procedures at the participant laboratories because the high *Cryptosporidium* counts occurred in four different laboratories as well on a trip control sample, and no oocysts or cysts were detected in any double-blind blank samples. Although the cause of the problem was not identified, EPA believes that the problem with the excessive number of oocysts was restricted to these five samples based on the results from all other valid *Cryptosporidium* and *Giardia* samples in the study, which were within the expected recovery range. Therefore, results for these samples were eliminated.

Three of the 11 laboratories in the study exercised the option in the Method to perform two acid dissociation steps after IMS. The combined counts from these two dissociations were used to determine the recovery for these laboratories.

SECTION 5 RESULTS

5.1 Reagent Water Results

A summary of the *Cryptosporidium* recovery results for the valid spiked reagent water analyses conducted by participant laboratories in the Study is provided in **Table 10**; a summary of the *Giardia* results for the valid spiked reagent water sample analyses is provided in **Table 11**. Recoveries of each organism are calculated based on the continuing calibration sample results generated by the referee laboratory concurrent with the preparation of the spiking suspensions distributed to the participant laboratories.

No oocysts were detected in any of the double-blind reagent water blank samples, and no non-detects occurred in the spiked samples. All laboratories examined 100% of the sample for reagent water samples. Raw data for reagent water sample analyses are provided in Appendix B to this report.

Table 10. Spiked Reagent Water Sample Analysis Results for *Cryptosporidium*

Lab	Spike level[1]	Recoveries by sample				Mean	SD	RSD
		1	2	3	4			
1	129.2 oocysts	37.2%	25.5%	31.0%	45.7%	34.8%	8.6%	24.8%
2	129.2 oocysts	24.0%	Invalid[2]	43.3%	28.6%	32.0%	10.1%	31.6%
3	129.2 oocysts	Invalid[3]						
4	129.2 oocysts	51.9%	47.2%	56.5%	58.8%	53.6%	5.1%	9.6%
5	129.2 oocysts	Invalid[4]						
6	129.2 oocysts	35.6%	32.5%	28.6%	Invalid[5]	32.2%	3.5%	10.8%
7	129.2 oocysts	27.1%	21.7%	13.9%	24.8%	21.9%	5.7%	26.2%
8	129.2 oocysts	40.2%	58.0%	53.4%	61.1%	53.2%	9.2%	17.3%
9	129.2 oocysts	Invalid[6]						
10	129.2 oocysts	29.4%	Invalid[2]	56.5%	19.3%	35.1%	19.2%	54.8%
11	129.2 oocysts	65.0%	57.3%	53.4%	44.9%	55.1%	8.4%	15.2%
Mean of all valid reagent water sample recoveries						40.4%		
Mean of the RSDs within each laboratory						23.8%		

[1]Spike level was determined from the results of the calibration samples prepared on February 17, 1999 (Table 6)
[2]The *Cryptosporidium* result for these samples were not used in data analysis because the number of oocysts counted greatly exceeded the estimated number of oocysts spiked (see Section 4 for details)
[3]All results from this laboratory were considered invalid because spiking suspensions warmed to just below room temperature after being subjected to unrefrigerated storage over a weekend
[4]All results from this laboratory were considered invalid due to improper aspiration and problems with the antibody staining kit
[5]This sample result was considered invalid because the concentrate for this sample was spilled during IMS, resulting in potential organism losses
[6]All results from this laboratory were considered invalid because samples were processed without *Giardia* IMS beads

Valid spiked reagent water results for *Cryptosporidium* were subjected to outlier analysis and additional data evaluation before being used to develop quality control (QC) acceptance criteria for

Cryptosporidium initial precision and recovery (IPR) and ongoing precision and recovery (OPR) tests for Method 1623.

Table 11. Spiked Reagent Water Sample Analysis Results for *Giardia*

Lab	Spike level[1]	Recoveries by sample				Mean	SD	RSD
		1	2	3	4			
1	129.4 cysts	29.4%	37.9%	30.9%	37.1%	33.8%	4.3%	12.7%
2	129.4 cysts	58.7%	23.2%	40.2%	21.6%	35.9%	17.4%	48.3%
3	129.4 cysts	Invalid[2]						
4	129.4 cysts	35.5%	41.0%	35.5%	34.0%	36.5%	3.1%	8.4%
5	129.4 cysts	Invalid[3]						
6	129.4 cysts	44.0%	35.5%	25.5%	Invalid	35.0%	9.3%	26.5%
7	129.4 cysts	19.3%	6.2%	3.9%	10.0%	9.9%	6.8%	69.1%
8	129.4 cysts	51.8%	50.2%	46.4%	35.5%	46.0%	7.3%	15.9%
9	129.4 cysts	Invalid[4]						
10	129.4 cysts	23.2%	34.0%	40.2%	19.3%	29.2%	9.6%	33.0%
11	129.4 cysts	68.0%	76.5%	67.2%	95.1%	76.7%	12.9%	16.9%
Mean of all valid reagent water sample recoveries						38.0%		
Mean of the RSDs within each laboratory						28.9%		

[1]Spike level was determined from the results of the calibration samples prepared on February 17, 1999 (Table 6)
[2]All results from this laboratory were considered invalid because spiking suspensions warmed to just below room temperature after being subjected to unrefrigerated storage over a weekend
[3]All results from this laboratory were considered invalid due to improper aspiration and problems with the antibody staining kit.
[4]All results from this laboratory were considered invalid because samples were processed without *Giardia* IMS beads.

Valid spiked reagent water results for *Giardia* were subjected to outlier analysis and additional data evaluation before being used to develop QC acceptance criteria for *Giardia* IPR and OPR tests for Method 1623.

5.2 Raw Surface Water Results

A summary of the *Cryptosporidium* recovery results for the valid spiked raw surface water analyses conducted by participant laboratories is provided in **Table 12**; a summary of the *Giardia* results for the valid spiked raw surface water sample analyses is provided in **Table 13**. Recoveries of each organism are calculated based on the continuing calibration results generated by the referee laboratory throughout spiking suspension generation. Raw data for raw surface water sample analyses are provided in Appendix C to this report.

Both oocysts and cysts were detected in unspiked raw surface water samples in two laboratories, and oocysts were detected in a third (Laboratory 9, which failed to use *Giardia* IMS beads). In these cases, the total number of each organism counted in the spiked raw surface water samples was reduced by the number of organisms detected in the unspiked raw surface water sample, per Section 9.5.1.3 of the Method, and recoveries were calculated based on this corrected count.

Sample concentrates at all but one laboratory yielded packed pellet volumes of ≤0.5 mL, the maximum pellet volume that can be processed through a single IMS procedure. Sample concentrates at Laboratory 3 yielded 1.5-mL packed pellets, but because Method 1623 includes an optional procedure for full sample analysis in these cases, and because this option was required for the Study, this laboratory analyzed the entire sample. As a result, no corrected counts were required to determine recovery.

Table 12. Spiked Raw Surface Water Sample Analysis Results for *Cryptosporidium*

Lab	Spike level[1]	Turbidity in NTU[2]	Oocysts detected in unspiked sample[3]	Recoveries for spiked samples		Mean	SD	RPD
				1	2			
1	129.2 oocysts	13.8	0	51.9%	44.1%	48.0%	5.5%	16.1%
2	129.2 oocysts	1.03	0	42.6%	49.5%	46.1%	4.9%	15.1%
3	129.2 oocysts	Invalid[4]						
4	129.2 oocysts	7.9	0	Invalid[5]	69.7%	69.7%	na	na
5	129.2 oocysts	Invalid[6]						
6	129.2 oocysts	1.4	0	20.1%	18.6%	19.3%	1.1%	8.0%
7	129.2 oocysts	2.3	3	15.5%	13.2%	14.3%	1.6%	16.2%
8	129.2 oocysts	13	1	Invalid[5]	51.1%	51.1%	na	na
9	129.2 oocysts	Invalid[7]						
10	129.2 oocysts	1.5	0	42.6%	15.5%	29.0%	19.2%	93.3%
11	129.2 oocysts	2.5	0	31.0%	61.9%	46.4%	21.9%	66.7%
Mean of all valid source water sample recoveries						37.7%		
Mean of the RPDs within each laboratory						35.9%		

[1]Spike level was determined from the results of the calibration samples prepared on February 17, 1999 (Table 6)
[2]Each set of raw surface water samples was collected in February 1999 from a surface water body serving as the source water for a drinking water utility
[3]The number of oocysts counted in the spiked raw surface water samples was reduced by the number of oocysts detected in the unspiked sample
[4]All results from this laboratory were considered invalid because spiking suspensions warmed to just below room temperature after being subjected to unrefrigerated storage over a weekend
[5]The *Cryptosporidium* result for these samples were not used in data analysis because the number of oocysts counted greatly exceeded the estimated number of oocysts spiked (see Section 4 for details)
[6]All results from this laboratory were considered invalid due to improper aspiration and problems with the antibody staining kit
[7]All results from this laboratory were considered invalid because samples were processed without *Giardia* IMS beads

Valid raw surface water results for *Cryptosporidium* were subjected to outlier analysis and additional data evaluation before being used to develop precision and recovery QC acceptance criteria for *Cryptosporidium* matrix spike/matrix spike duplicate (MS/MSD) test for Method 1623.

Table 13. Spiked Raw Surface Water Sample Analysis Results for *Giardia*

Lab	Spike level[1]	Turbidity in NTU[2]	Cysts detected in unspiked sample[3]	Recoveries for spiked samples		Mean	SD	RPD
				1	2			
1	129.4 cysts	13.8	0	66.5%	79.6%	73.0%	9.3%	18.0%
2	129.4 cysts	1.03	0	31.7%	35.5%	33.6%	2.7%	11.5%
3	129.4 cysts	Invalid[4]						
4	129.4 cysts	7.9	0	34.8%	38.6%	36.7%	2.7%	10.5%
5	129.4 cysts	Invalid[5]						
6	129.4 cysts	1.4	0	22.4%	20.9%	21.6%	1.1%	7.1%
7	129.4 cysts	2.3	1	3.1%	14.7%	8.9%	8.2%	130.4%
8	129.4 cysts	13	6	65.7%	58.7%	62.2%	4.9%	11.2%
9	129.4 cysts	Invalid[6]						
10	129.4 cysts	1.5	0	43.3%	23.2%	33.2%	14.2%	60.5%
11	129.4 cysts	2.5	0	64.1%	71.1%	67.6%	4.9%	10.3%
Mean of all valid source water sample recoveries						42.1%		
Mean of the RPDs within each laboratory						32.4%		

[1]Spike level was determined from the results of the calibration samples prepared on February 17, 1999 (Table 6)
[2]Each set of raw surface water samples was collected in February 1999 from a surface water body serving as the source water for a drinking water utility
[3]The number of oocysts recovered from a sample was reduced by the number of oocysts detected in the unspiked source water
[4]All results from this laboratory were considered invalid because spiking suspensions warmed to just below room temperature after being subjected to unrefrigerated storage over a weekend
[5]All results from this laboratory were considered invalid due to improper aspiration and problems with the antibody staining kit
[6]All results from this laboratory were considered invalid because samples were processed without *Giardia* IMS beads

Valid raw surface water results for *Giardia* were subjected to outlier analysis and additional data evaluation before being used to develop precision and recovery QC acceptance criteria for *Giardia* matrix spike/matrix spike duplicate (MS/MSD) test for Method 1623.

SECTION 6 DATA DISCUSSION AND ANALYSIS

6.1 Discussion

The results of the ongoing flow cytometer calibration samples (Table 6), demonstrate that the number of oocysts and cysts in each spiking suspension used in the Study could be estimated with a high degree of precision. The results of the trip control tests for *Giardia* demonstrate that an acceptable percentage of organisms was transferred from the spiking suspension containers after the spiking suspensions were shipped from the referee laboratory to the participant laboratories. Although the overall results of the *Cryptosporidium* trip control samples similarly demonstrate an acceptable transfer rate for this organism, the results for trip control sample 4 exceeded the estimated spike dose. This problem also occurred in one sample in each of four laboratories (two reagent water samples and two raw surface water samples). The *Giardia* counts in these samples were not disparate from the counts in all other samples in the Study. Although the cause of the excessive number of oocysts in these five samples could not be determined by the referee laboratory, SCC, or EPA, the Agency believes that the problem was restricted to these five samples based on the results from all other valid *Cryptosporidium* and *Giardia* samples in the study, which were within the expected recovery range.

A summary of the overall precision and recovery for Method 1623 is provided in **Table 14**. These summary data include all valid results, before outlier analyses. No statistically significant difference was found between the mean *Cryptosporidium* recoveries and the mean *Giardia* recoveries using Method 1623, based on a two-sample t-test at α=.05 (reagent: t=-0.54 p=.59; raw surface water: t=-0.58 p=.57). Similarly, no statistically significant difference was found between the total variances (combined within-laboratory and between-laboratory) of *Cryptosporidium* and *Giardia* recoveries, based on an F-test at α=.05 (reagent: F=1.9 p=.10; raw surface water: F=1.5 p=.47).

Table 14. Summary *Cryptosporidium* and *Giardia* Results for Method 1623

Matrix	Organism	Mean Recovery	Mean RSD or RPD
Reagent water	*Cryptosporidium*	40%	24%
	Giardia	38%	29%
Source water	*Cryptosporidium*	38%	36%
	Giardia	42%	32%

Regarding the *Cryptosporidium* results, one of the concerns with the use of the combined *Cryptosporidium/Giardia* IMS step was the potential for lower *Cryptosporidium* recoveries than when IMS is used to purify sample concentrates for that organism alone. The *Cryptosporidium* results from the Method 1623 round robin are compared to those of the Method 1622 round robin in **Table 15.**

Table 15. Comparison of Method 1622 and Method 1623 *Cryptosporidium* Results

Matrix	Statistic	Method 1622	Method 1623
Reagent water	Mean recovery	35%	40%
	Mean RSD	30%	24%
Raw surface water	Mean recovery	43%	38%
	Mean RPD	56%	36%

No statistically significant difference was found between the mean Method 1622 *Cryptosporidium* recoveries and the mean Method 1623 *Cryptosporidium* recoveries based on a two-sample t-test at α=.05 (reagent: t=-1.5 p=.14; raw surface water: t=0.78 p=.44). Similarly, no statistically significant difference was found between the total variances (combined within-laboratory and between-laboratory) of Method 1622 and 1623 recoveries, based on an F-test at α=.05 (reagent: F=1.0 p=.89; raw surface water: F=1.5 p=.44). The results of this test demonstrates that the performance of Methods 1622 and 1623 are equivalent for *Cryptosporidium*, and that the addition *Giardia* as a target analyte does not have an adverse effect on the performance of the method for *Cryptosporidium*.

6.2 Data Analysis

Acceptable sample results submitted by the participant laboratories (Section 5) were screened for outliers in accordance with laboratory ranking and individual-point procedures described in American Society for Testing and Materials (ASTM) guidance[4]. Laboratories were first ranked and screened according to Youden's test for outlying laboratories to identify laboratories with significantly higher or lower results than the other laboratories. Individual sample results were then screened for outliers using Grubb's test for outlying observations. The remaining results were then evaluated against the existing QC acceptance criteria published in Method 1623.

6.2.1 Youden's Test

Youden's test was performed on data from all laboratories but Laboratories 3, 5, and 9. The data from laboratory 3 were considered invalid because spiking suspensions warmed to just below room temperature after being subjected to unrefrigerated storage over a weekend. The data from Laboratory 5 were considered unacceptable due to potential organism losses during aspiration and problems with the staining kit that was used. The data from Laboratory 9 were considered unacceptable because only the *Cryptosporidium* portion of the IMS procedure was followed, rather than the combined *Cryptosporidium/Giardia* IMS procedure. Because Youden's test cannot be run if all laboratories do not have the same number of results, the invalid results from the four laboratories that indicated very high numbers of oocysts were replaced by the mean recovery of the remaining *Cryptosporidium* results in each laboratory for that matrix to run Youden's test.

Laboratories were ranked according to the magnitude of the difference between the organisms counted by a single laboratory and the mean count across all laboratories for the corresponding sample type. Sample results for each laboratory were ordered based on counts, such that the replicate with the lowest count for a specific lab, matrix, and organism, would be compared to the replicates from the other labs with the lowest concentration for that matrix and organism, and so forth. Laboratory 7 was rejected for both *Cryptosporidium* and *Giardia* following application of the Youden laboratory ranking test at the 5% significance level using this sample ordering scheme.

6.2.2 Grubb's Test

After the outlier laboratory data were removed, the remaining valid reagent water sample results (25 *Cryptosporidium* and 27 *Giardia*) and raw surface water results (12 *Cryptosporidium* and 14 *Giardia*) were screened for outliers using Grubb's test for outlying observations. One *Giardia* reagent water sample result (Sample 4 from Laboratory 2 in Table 11) was rejected following application of the Grubb's test. This data point fell outside the critical value of 2.86 standard deviations from the mean

[4]Standard Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D-19 on Water (ASTM D2777-96), September 1996.

result of all reagent water *Giardia* samples. Grubb's test also was run after the natural logarithmic transformation had been applied to the data. In this case, no results were identified as being outliers.

6.2.3 Evaluation Against Existing Acceptance Criteria

After applying the ASTM laboratory ranking and individual-point procedures to the data generated through the Study, the individual raw surface water results for *Cryptosporidium* were evaluated against the existing matrix spike (MS) recovery criteria published in the January 1999 version of Method 1622 to ensure that samples subject to unacceptable matrix interferences were not included in the development of final QC acceptance criteria for Method 1623. This evaluation did not apply to *Giardia* results, as no *Giardia* criteria had been developed prior to the Study. The existing *Cryptosporidium* criteria specify that MS recoveries must fall between 13% and 143%. All results fell within the QC acceptance criteria; therefore, no results were removed.

SECTION 7 DEVELOPMENT OF QC ACCEPTANCE CRITERIA

Quality control (QC) acceptance criteria for Method 1623 were developed using the applicable procedures in Chapter 3 of EPA's *Guide to Method Flexibility and Approval of EPA Water Methods*[5]. Development of these criteria is detailed in Sections 7.1 and 7.2.

7.1 Initial Precision and Recovery and Ongoing Precision and Recovery

QC acceptance criteria for initial precision and recovery (IPR) and ongoing precision and recovery (OPR) were developed for each organism using the 25 spiked *Cryptosporidium* and 26 spiked *Giardia* reagent water results remaining after data validation (Section 5) and outlier analysis and evaluation against existing criteria (Section 6). QC acceptance criteria were calculated using the recoveries and a linear combination of between-laboratory and within-laboratory variability. Examination of the distributions of the *Cryptosporidium* and *Giardia* results through graphical analysis and the Shapiro-Wilk test did not discern whether the data fit a normal or log-normal distribution. Precision criteria were calculated using non-transformed data, and recovery criteria were calculated using natural log-transformed data to establish criteria that more consistently reflect the results of the individual laboratories and previous criteria.

The pooled within-laboratory standard deviation (s_w) was calculated as follows:

$$s_w = \sqrt{\frac{1}{(n_T - m)} \sum_{i \in (1,\ldots,m)} ((n_i - 1) * s_i^2)}$$

Where:
n_i = *the number of spiked reagent water samples taken by lab i for that organism,*
s_i = *the standard deviation of those samples,*
m = *the number of labs (7 after data validation and outlier analysis), and*
n_T = *the total number of reagent water samples from all labs for the corresponding organism.*

The IPR QC acceptance criterion for precision as relative standard deviation (RSD) was calculated as:

$$RSD_{MAX} = \sqrt{F_{(.95;\ 3,\ n_T - m)}} * \frac{s_w}{X_{mean}}$$

[5]EPA Guide to Method Flexibility and Approval of EPA Water Methods, EPA 821-D-96-004, December 1996.

Where:
$F_{(.95;3,nT-m)}$ *= the 95th percentile of an F distribution with 3 and* n_T*-m degrees of freedom,*
$\overline{X}_{mean}$ *= the mean of all* $\overline{X}_i$*,*
$\overline{X}_i$ *= the mean of spiked reagent water samples for that organism in lab i, and*
m = the number of laboratories (7 after data validation and outlier analysis).

The IPR and OPR acceptance criteria for recovery were calculated after the natural logarithmic transformation was applied to the data, and using the equations as follows:

The pooled within-laboratory standard deviation (s_w) was calculated as follows:

$$s_{Log,w} = \sqrt{\frac{1}{(n_T - m)} \sum_{i \in (1,\ldots,m)} ((n_i - 1) * s^2_{Log,i})}$$

Where:
n_i *= the number of spiked reagent water samples taken by lab i for that organism,*
$s\ Log_{,i}$ *= the standard deviation of those samples,*
m = the number of labs (7 after data validation and outlier analysis), and
n_T *= the total number of reagent water samples from all labs for the corresponding organism (*n_T*=27 for* Giardia *because sample X was not identified as an outlier after log-transformation).*

The between-laboratory standard deviation (s_b) was calculated as follows:

$$s_b = \sqrt{\frac{1}{m-1} \sum_{i \in (1,\ldots,m)} (\overline{Y}_i - Y_{mean})^2}$$

Where:
$\overline{Y}_{mean}$ *= the mean of all* $\overline{Y}_i$*,*
$\overline{Y}_i$ *= the mean of the log-transformed spiked reagent water samples for that organism in lab i, and*
m = the number of laboratories (7 after data validation and outlier analysis).

The combined standard deviation (s_c) for IPR is:

$$s_c = \sqrt{(1 + \frac{1}{m}) s_b^2 + (\frac{1}{4} - \frac{1}{n}) s^2_{Log,w}}$$

Where:

$\bar{n} = \frac{1}{m}\sum_{i\in(1,..,m)} n_i$ *(the mean number of reagent results per lab) and*

m=the number of labs (7 after data validation and outlier analysis).

Upper and lower limits for IPR samples were then calculated as:

$$EXP(Y_{mean} \pm t_{(.975;\ m)} * s_c)$$

The combined standard deviation (s_c) for OPR is:

$$s_c = \sqrt{(1+\frac{1}{m})s_b^2 + (1-\frac{1}{n})s_{Log,w}^2}$$

Where:
$\bar{n}$ = the mean number of spiked reagent water samples per lab (as calculated above), and
m = the number of labs (7 after data validation and outlier analysis).

Upper and lower limits for OPR samples were then calculated as:

$$EXP(Y_{mean} \pm t_{(.975;\ 2*m)} * s_c)$$

The resulting *Cryptosporidium* upper acceptance limits for recovery for IPR and OPR were 82% and 90%, respectively. The resulting *Giardia* upper acceptance limits for recovery for IPR and OPR were 90% and 96%, respectively. In recognition of the demonstrated ability of laboratories to achieve continually improved recoveries over time using Methods 1622 and 1623, and in recognition of the advances that are occurring in analytical technology, the upper acceptance limits for IPR and OPR recovery were set at 100%. Setting the upper acceptance limits at 100% prevents laboratory data from being flagged or qualified by data reviewers and data users when laboratories achieve mean recoveries as high as 100%.

The final IPR and OPR QC acceptance criteria are listed in **Table 16**.

Table 16. Initial and Ongoing Precision and Recovery Criteria

Organism	Test	Precision as RSD_{max}	Recovery	
			Lower Acceptance Limit	Upper Acceptance Limit
Cryptosporidium	IPR	40%	21%	100%
	OPR	NA	19%	100%
Giardia	IPR	41%	17%	100%
	OPR	NA	16%	100%

7.2 Matrix Spike/Matrix Spike Duplicate Recovery and Relative Percent Difference

QC acceptance criteria for matrix spike/matrix spike duplicate (MS/MSD) precision and recovery were developed using the spiked raw surface water results remaining after data validation (Section 5) and outlier analysis (Section 6). Examination of the distributions of the *Cryptosporidium* and *Giardia* results through graphical analysis and the Shapiro-Wilk test did not discern whether the data fit a normal or log-normal distribution. Consistent with the IPR/OPR specifications, precision criteria were calculated using non-transformed data and recovery criteria were calculated using natural log-transformed data in order to establish criteria that more consistently reflect the results of the individual laboratories and previous criteria.

The QC acceptance criterion for MS/MSD precision (as relative percent difference [RPD]) was developed as follows:

The overall mean percent recovery (X_{mean}) was calculated as the average of the percent recoveries of the 12 Cryptosporidium raw surface water and 14 Giardia sample results, calculated as follows:

$$X_{mean} = \sqrt{\frac{1}{n_T}\sum_{i\in(1,\ldots,m)}\sum_{j\in(1,\ldots,2)} X_{ij}}$$

Where:
n_T=the total number of spiked source water samples for the corresponding organism,
X_{ij}=the jth spiked source water sample for the organism for laboratory I, and
m = the number of labs (7 after data validation and outlier analysis).

The pooled within-lab standard deviation (s_w) was calculated as follows:

$$s_w = \sqrt{\frac{1}{(n_T - m)}\sum_{i\in(1,\ldots,m)} ((n_i - 1) * s_i^2)}$$

Where:
s_i = *the standard deviation of the spiked source water samples for laboratory i, and*
m = *the number of labs (the number of laboratories with two spiked source water samples after data validation and outlier analysis: 5 for* Cryptosporidium *and 7 for* Giardia*).*

The RSD was calculated by dividing the pooled within-laboratory standard deviation (s_w) of the raw water sample results by X_{mean}. The RSD was then multiplied by 2, to calculate the QC acceptance criterion for precision (RPD_{max}) in the MS/MSD test as follows:

$$RPD_{max}(\%)=2 * RSD$$

The QC acceptance criteria for recovery were calculated as follows:

The mean log percent recovery ($\overline{Y}$) was calculated as the average of the log percent recovery of the raw surface water sample results. The total standard deviation (s_y) was calculated as the standard deviation of all log percent recoveries.

The QC acceptance criteria for recovery was calculated by constructing a $\pm$ 2.2 s_y window around the average log percent recovery, $\overline{Y}$,using the total standard deviation, s_y, and then exponentiating the limits to return to the original scale.

The final MS/MSD acceptance criteria are listed in **Table 17**.

Table 17. Matrix Spike/Matrix Spike Duplicate Criteria

Organism	Test	Precision as RPD_{max}	Recovery	
			Lower Acceptance Limit	Upper Acceptance Limit
Cryptosporidium	MS/MSD	61%	13%	111%
Giardia	MS/MSD	30%	15%	118%

Because Method 1623 requires MS and MSD samples to be analyzed in conjunction with an identical, unspiked raw surface water sample, which may contain background organisms, the formula used to determine percent recovery of the spiked oocysts or cysts is:

$$\% \text{ Recovery} = 100 \frac{(A-B)}{T}$$

Where:
A = *number of oocysts or cysts counted in MS sample*
B = *number of oocysts or cysts counted in unspiked sample*
T = *true number of oocysts or cysts in the spiking suspension*

During routine sample analysis, Method 1623 requires laboratories to perform only MS tests, rather than MS/MSD tests. (The MS/MSD test in Method 1623 is required to demonstrate that method modifications produce results equal or superior to results produced by the unmodified Method.) Therefore, for routine use, Method 1623 requires that the MS result meet the lower and upper acceptance limits for recovery in the MS/MSD test, but does not apply a precision specification.

SECTION 8 CONCLUSION AND DISCUSSION

Results of this Study demonstrate that EPA Method 1623 is valid for use in determining the concentration of *Cryptosporidium* oocysts and *Giardia* cysts in raw surface water. Results of the Study enabled assessment of the Method's performance in reagent water and raw surface waters, and development of quality control (QC) acceptance criteria that will be used to confirm acceptable laboratory and Method performance on an ongoing basis in surface water monitoring surveys and other studies.

As discussed in Section 6, there was no statistically significant difference between the *Cryptosporidium* and *Giardia* results in either of the matrices tested in this Study. In addition, *Cryptosporidium* precision and recovery results using Method 1623 were not statistically significantly different from those generated using Method 1622 in EPA's August 1998 interlaboratory validation study. This indicates that *Cryptosporidium* recovery is not adversely impacted when combined with simultaneous *Giardia* recovery during the immunomagnetic separation step.

Method 1623 was revised in April 1999 to include the final QC acceptance criteria and comments and recommendations provided by laboratories participating in the Study. With the results of this Study, EPA Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA (EPA-821-R-99-006) can be considered valid for the determination of *Cryptosporidium* and *Giardia* in water, and EPA recommends the use of either Method 1622 or 1623, depending on the need to monitor *Cryptosporidium* only or *Cryptosporidium* and *Giardia* simultaneously.

APPENDIX A

Technical Clarifications to the December 1998 Draft of Method 1623 Implemented in the Interlaboratory Validation of Method 1623

14.0 Sample Staining

14.1 Prepare positive and negative controls.

14.1.1 For the positive control, pipette 10 μL of positive antigen or 200 to 500 intact oocysts and 200 to 500 cysts to the center of a well.

14.1.2 For the negative control, pipette 50 μL of 150 mM PBS into the center of a well and spread it over the well area with a pipette tip.

14.1.3 Air-dry the control slides.

14.2 Apply one drop of Detection Reagent (from direct labeling kit, Section 7.7) to each well.

14.3 Apply one drop of Counterstain (from direct labeling kit, Section 7.7) to each well.

14.4 Spread over entire well with applicator stick, if necessary. Do not allow the stick to scratch the treated surface of the slide. Use a different applicator stick for each slide.

14.5 Place the slides in a humid chamber in the dark and incubate at room temperature for approximately 30 minutes. The humid chamber consists of a tightly sealed plastic container containing damp paper towels on top of which the slides are placed.

14.6 Apply one drop of 1X Wash Buffer (made from 20X concentrate in direct labeling kit, Section 7.7) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess Detection Reagent and Counterstain from below the well using a clean Pasteur pipette. Avoid disturbing the sample.

NOTE: Do not allow slides to dry.

14.7 Apply 50 μL of 4',6-diamidino-2-phenylindole (DAPI) staining solution (Section 7.8.2) to each well. Allow to stand at room temperature for approximately 1 minute.

14.8 Apply one drop of 1X Wash Buffer (from direct labeling kit, Section 7.7) to each well. Tilt each slide on a clean paper towel, long edge down. Gently aspirate the excess DAPI staining solution from below the well using a clean Pasteur pipette. Avoid disturbing the sample.

NOTE: Do not allow slides to dry.

14.9 Add one drop of Mounting Medium (from direct labeling kit, Section 7.7) to each well.

14.10 Apply a cover slip. Use a tissue to remove excess mounting fluid from the edges of the coverslip.

14.11 Record the date and time that staining was completed on the bench sheet. If slides will not be read immediately, store in a humid chamber in the dark at 0°C to 8°C until ready for examination.

APPENDIX B

Raw *Cryptosporidium* Recovery Data from Analysis of Reagent Water Samples

Laboratory	Sample	Spike Level (Oocysts)	Oocysts Counted[1]	Recovery	Valid Result?
1	A	129.2	48	37.2%	Yes
1	B	129.2	33	25.5%	Yes
1	C	129.2	40	31.0%	Yes
1	D	Blank	0	NA	Yes
1	E	129.2	59	45.7%	Yes
2	A	129.2	31	24.0%	Yes
2	B	129.2	161	124.6%	No
2	C	129.2	56	43.3%	Yes
2	D	Blank	0	NA	Yes
2	E	129.2	37	28.6%	Yes
3	A	129.2	27	20.9%	No
3	B	129.2	14	10.8%	No
3	C	129.2	35	27.1%	No
3	D	Blank	0	NA	No
3	E	129.2	18	13.9%	No
4	A	129.2	67	51.9%	Yes
4	B	129.2	61	47.2%	Yes
4	C	129.2	73	56.5%	Yes
4	D	Blank	0	NA	Yes
4	E	129.2	76	58.8%	Yes
5	A	129.2	1	0.8%	No
5	B	129.2	2	1.5%	No
5	C	129.2	8	6.2%	No
5	D	Blank	0	NA	No
5	E	129.2	0	0.0%	No
6	A	129.2	46	35.6%	Yes
6	B	129.2	42	32.5%	Yes
6	C	129.2	37	28.6%	Yes
6	D	Blank	0	NA	Yes
6	E	129.2	7	5.4%	No
7	A	129.2	35	27.1%	Yes
7	B	129.2	28	21.7%	Yes
7	C	129.2	18	13.9%	Yes
7	D	Blank	0	NA	Yes
7	E	129.2	32	24.8%	Yes

Raw *Cryptosporidium* Recovery Data from Analysis of Reagent Water Samples

Laboratory	Sample	Spike Level (Oocysts)	Oocysts Counted[1]	Recovery	Valid Result?
8	A	129.2	52	40.2%	Yes
8	B	129.2	75	58.0%	Yes
8	C	129.2	69	53.4%	Yes
8	D	Blank	0	NA	Yes
8	E	129.2	79	61.1%	Yes
9	A	129.2	33	25.5%	No
9	B	129.2	33	25.5%	No
9	C	129.2	27	20.9%	No
9	D	Blank	0	NA	No
9	E	129.2	26	20.1%	No
10	A	129.2	38	29.4%	Yes
10	B	129.2	292	226.0%	No
10	C	129.2	73	56.5%	Yes
10	D	Blank	0	NA	Yes
10	E	129.2	25	19.3%	Yes
11	A	129.2	84	65.0%	Yes
11	B	129.2	74	57.3%	Yes
11	C	129.2	69	53.4%	Yes
11	D	Blank	0	NA	Yes
11	E	129.2	58	44.9%	Yes

[1]Based on examination of 100% of each sample

APPENDIX C

Raw *Giardia* Recovery Data from Analysis of Reagent Water Samples

Laboratory	Sample	Spike Level (Cysts)	Cysts Counted[1]	Recovery	Valid Result?
1	A	129.4	38	29.4%	Yes
1	B	129.4	49	37.9%	Yes
1	C	129.4	40	30.9%	Yes
1	D	Blank	0	NA	Yes
1	E	129.4	48	37.1%	Yes
2	A	129.4	76	58.7%	Yes
2	B	129.4	30	23.2%	Yes
2	C	129.4	52	40.2%	Yes
2	D	Blank	0	NA	Yes
2	E	129.4	28	21.6%	Yes
3	A	129.4	16	12.4%	No
3	B	129.4	19	14.7%	No
3	C	129.4	34	26.3%	No
3	D	Blank	0	NA	No
3	E	129.4	29	22.4%	No
4	A	129.4	46	35.5%	Yes
4	B	129.4	53	41.0%	Yes
4	C	129.4	46	35.5%	Yes
4	D	Blank	0	NA	Yes
4	E	129.4	44	34.0%	Yes
5	A	129.4	15	11.6%	No
5	B	129.4	23	17.8%	No
5	C	129.4	16	12.4%	No
5	D	Blank	0	NA	No
5	E	129.4	17	13.1%	No
6	A	129.4	57	44.0%	Yes
6	B	129.4	46	35.5%	Yes
6	C	129.4	33	25.5%	Yes
6	D	Blank	0	NA	Yes
6	E	129.4	38	29.4%	No
7	A	129.4	25	19.3%	Yes
7	B	129.4	8	6.2%	Yes
7	C	129.4	5	3.9%	Yes
7	D	Blank	0	NA	Yes
7	E	129.4	13	10.0%	Yes

Raw *Giardia* Recovery Data from Analysis of Reagent Water Samples

Laboratory	Sample	Spike Level (Cysts)	Cysts Counted[1]	Recovery	Valid Result?
8	A	129.4	67	51.8%	Yes
8	B	129.4	65	50.2%	Yes
8	C	129.4	60	46.4%	Yes
8	D	Blank	0	NA	Yes
8	E	129.4	46	35.5%	Yes
9	A	129.4	0	0.0%	No
9	B	129.4	0	0.0%	No
9	C	129.4	0	0.0%	No
9	D	Blank	0	NA	No
9	E	129.4	0	0.0%	No
10	A	129.4	30	23.2%	Yes
10	B	129.4	44	34.0%	Yes
10	C	129.4	52	40.2%	Yes
10	D	Blank	0	NA	Yes
10	E	129.4	25	19.3%	Yes
11	A	129.4	88	68.0%	Yes
11	B	129.4	99	76.5%	Yes
11	C	129.4	87	67.2%	Yes
11	D	Blank	0	NA	Yes
11	E	129.4	123	95.1%	Yes

[1]Based on examination of 100% of each sample

APPENDIX D

Raw *Cryptosporidium* Recovery Data from Analysis of Raw Surface Water Samples

Laboratory	Sample	NTU	Spike level (Oocysts)	Oocysts Counted[1]	Corrected count[2]	Recovery	Valid Result?
1	F	13.8	129.2	67	67	51.9%	Yes
1	G	13.8	129.2	57	57	44.1%	Yes
1	Unspiked	13.8	Unspiked	0	NA	NA	Yes
2	F	1.03	129.2	55	55	42.6%	Yes
2	G	1.03	129.2	64	64	49.5%	Yes
2	Unspiked	1.03	Unspiked	0	NA	NA	Yes
3	F	3	129.2	27	27	20.9%	No
3	G	3	129.2	28	28	21.7%	No
3	Unspiked	3	Unspiked	0	NA	NA	No
4	F	7.9	129.2	216	216	167.2%	No
4	G	7.9	129.2	90	90	69.7%	Yes
4	Unspiked	7.9	Unspiked	0	NA	NA	Yes
5	F	0.98	129.2	5	5	3.9%	No
5	G	0.98	129.2	6	6	4.6%	No
5	Unspiked	0.98	Unspiked	0	NA	NA	No
6	F	1.4	129.2	26	26	20.1%	Yes
6	G	1.4	129.2	24	24	18.6%	Yes
6	Unspiked	1.4	Unspiked	0	NA	NA	Yes
7	F	2.3	129.2	23	20	15.5%	Yes
7	G	2.3	129.2	20	17	13.2%	Yes
7	Unspiked	2.3	Unspiked	3	NA	NA	Yes
8	F	13	129.2	370	369	285.6%	No
8	G	13	129.2	67	66	51.1%	Yes
8	Unspiked	13	Unspiked	1	NA	NA	Yes
9	F	6	129.2	30	29	22.4%	No
9	G	6	129.2	26	25	19.3%	No
9	Unspiked	6	Unspiked	1	NA	NA	No
10	F	1.5	129.2	55	55	42.6%	Yes
10	G	1.5	129.2	20	20	15.5%	Yes
10	Unspiked	1.5	Unspiked	0	NA	NA	Yes
11	F	2.5	129.2	40	40	31.0%	Yes
11	G	2.5	129.2	80	80	61.9%	Yes
11	Unspiked	2.5	Unspiked	0	NA	NA	Yes

[1]Based on examination of 100% of each sample

[2]The number of oocysts counted in each spiked sample was reduced by the number of environmental oocysts detected in the corresponding unspiked sample

APPENDIX E

Raw *Giardia* Recovery Data from Analysis of Raw Surface Water Samples

Laboratory	Sample	NTU	Spike level (Cysts)	Cysts Counted[1]	Corrected count[2]	Recovery	Valid Result?
1	F	13.8	129.4	86	86	66.5%	Yes
1	G	13.8	129.4	103	103	79.6%	Yes
1	Unspiked	13.8	Unspiked	0	NA	NA	Yes
2	F	1.03	129.4	41	41	31.7%	Yes
2	G	1.03	129.4	46	46	35.5%	Yes
2	Unspiked	1.03	Unspiked	0	NA	NA	Yes
3	F	3	129.4	26	26	20.1%	No
3	G	3	129.4	13	13	10.0%	No
3	Unspiked	3	Unspiked	0	NA	NA	No
4	F	7.9	129.4	45	45	34.8%	Yes
4	G	7.9	129.4	50	50	38.6%	Yes
4	Unspiked	7.9	Unspiked	0	NA	NA	Yes
5	F	0.98	129.4	46	46	35.5%	No
5	G	0.98	129.4	48	48	37.1%	No
5	Unspiked	0.98	Unspiked	0	NA	NA	No
6	F	1.4	129.4	29	29	22.4%	Yes
6	G	1.4	129.4	27	27	20.9%	Yes
6	Unspiked	1.4	Unspiked	0	NA	NA	Yes
7	F	2.3	129.4	5	4	3.1%	Yes
7	G	2.3	129.4	20	19	14.7%	Yes
7	Unspiked	2.3	Unspiked	1	NA	NA	Yes
8	F	13	129.4	91	85	65.7%	Yes
8	G	13	129.4	82	76	58.7%	Yes
8	Unspiked	13	Unspiked	6	NA	NA	Yes
9	F	6	129.4	0	0	0.0%	No
9	G	6	129.4	0	0	0.0%	No
9	Unspiked	6	Unspiked	0	NA	NA	No
10	F	1.5	129.4	56	56	43.3%	Yes
10	G	1.5	129.4	30	30	23.2%	Yes
10	Unspiked	1.5	Unspiked	0	NA	NA	Yes
11	F	2.5	129.4	83	83	64.1%	Yes
11	G	2.5	129.4	92	92	71.1%	Yes
11	Unspiked	2.5	Unspiked	0	NA	NA	Yes

[1]Based on examination of 100% of each sample
[2]The number of oocysts counted in each spiked sample was reduced by the number of environmental oocysts detected in the corresponding unspiked sample